REMARKS

Claims 1, 5, 45 and 49 have been amended herein. Claims 5 and 49 have been amended to correct clerical errors. Claims 1 and 45 have been amended to replace the phrase "pluripotent adult stem cells" with "non-embryonic pluripotent stem cells." Support for the amendment to claims 1 and 45 can be found throughout the specification and claims as originally filed, for example, in the specification at page 11, line 32 to page 12, line 8. Claims 12, 14-16, 18, 31-44, 53 and 67-68 have been previously cancelled. Claims 1-11, 13, 17, 19-30, 45-52, 54-66, 69 and 70 are pending in this application. No new matter has been added herein and entry thereof is respectfully requested.

Applicants address herein each issue raised in the Office Action of June 14, 2011.

I. The Rejection of Claims 1-11, 13, 17, 19-30, 45-52, 54-66, 69 and 70 under 35 U.S.C. § 112, Second Paragraph, as Indefinite Should be Withdrawn

The Office has rejected claims 1-11, 13, 17, 19-30, 45-52, 54-66, 69 and 70 under 35 U.S.C. § 112, second paragraph, as indefinite because allegedly the phrase "pluripotent adult stem cells" in claims 1 and 45 is vague. Office Action at page 2, last paragraph. According to the Office, the specification fails to provide a specific definition for "pluripotent adult stem cells" and it is unclear what kind of cells are considered "pluripotent adult stem cells." *Id.*

In response, Applicants note that the phrase "pluripotent adult stem cells" has been deleted from claims 1 and 45 and replaced with "non-embryonic pluripotent stem cells." Accordingly, the rejection of claims 1-11, 13, 17, 19-30, 45-52, 54-66, 69 and 70 as indefinite

has been rendered moot. Consequently, Applicants respectfully request withdrawal of the rejection.

II. The Rejection of Claims 1-11, 13, 17, 19-30, 45-52, 54-66, 69 and 70 under 35 U.S.C. § 112, First Paragraph, Written Description, Should be Withdrawn

The Office has rejected claims 1-11, 13, 17, 19-30, 45-52, 54-66, 69 and 70 under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. Office Action at page 3, paragraph no. 4. According to the Office, the phrase, "pluripotent adult stem cells" is considered new matter as the specification only states "adult stem cells" rather than "pluripotent adult stem cells." *Id.* at third paragraph. Consequently, the Office alleges that because "[t]here is no support for the phrase 'pluripotent adult stem cells' in the specification" the phrase is considered new matter. *Id.*

In the interest of expediting the prosecution of this application and without acquiescing to the rejection, Applicants have deleted the phrase "pluripotent adult stem cells" and instead have replaced it with the phrase "pluripotent non-embryonic stem cells." Consequently, the rejection has been rendered moot and reconsideration and withdrawal is respectfully requested.

To the extent that the rejection applies to amended claims 1 and 45, and the claims that depend therefrom, Applicants assert that these claims have sufficient written description based on the specification and what was known to one of skill in the art to reasonably convey that the inventors, at the time the application was filed, had possession of the claimed invention.

With regard to written description, "[t]he specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public. *In re Buchner,* 929 F.2d 660, 661, 18 USPQ2d 1331, 1332

(Fed. Cir. 1991); *Hybritech, Inc.* v. *Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384,231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied,* 480 U.S. 947 (1987); and *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F .2d 1452, 1463, 221 USPQ 481,489 (Fed. Cir. 1984). *See,* MPEP § 2164.05(a) (Emphasis added). The Federal Circuit also noted that the written description requirement does not require that "every invention must be described in the same way. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution." Slip op. at 17 (citing *Capon* v. *Eshhar,* 419 F.3d 1349, 1358 (Fed. Cir. 2005)). The court acknowledged that the scope of the written description for each application will vary because of the differences in the state of the knowledge in the field and in the predictability of the science. *Id.*

An adequate written description of the invention may be shown by any description of sufficient, relevant identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention. *See, e.g., Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1323, (Fed Cir. 2000). Moreover, the written description requirement does not require that the specification disclose *in haec verba* (verbatim) a phrase present in a claim but merely requires support in the specification through express, implicit, or inherent disclosure. *See* M.P.E.P. 2163(I)(B), Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, para. 1, "Written Description" Requirement.

Claims 1 and 45, as amended herein, are directed to a method for producing embryoid bodies from pluripotent cells...wherein the pluripotent cells are selected from the group consisting of embryonic stem (ES) cells, primordial germ (EG) cells and non-embryonic pluripotent stem cells. Accordingly, the claims have been amended to specify that, in addition to

embryonic stem (ES) cells and primordial germ (EG) cells, "non-embryonic pluripotent stem cells" are also included in the claimed methods for producing embryoid bodies.

Preliminarily, Applicants note that the specification defines "embryoid bodies" as being synonymous with "aggregate bodies" and indicates that the term refers to "aggregates of differentiated and undifferentiated cells that appear when *ES* cells overgrow in monolayer cultures, or are maintained in suspension cultures." Specification at page 6, lines 26-28. (emphasis added). The specification further defines ES cells as embryonic cells of various types in addition to "other types of pluripotent cells." *Id.* at page 6, lines 4-9. The specification defines the other types of pluripotent cells as "[a]ny cells of mammalian origin that are capable of producing progeny that are derivatives of all three germinal layers...*regardless of whether they were derived from embryonic tissue, fetal tissue or other sources*." *Id.* at lines 10-12. "Other sources" are clearly pluripotent cells which are of non-embryonic or non-fetal sources. Consequently, the specification clearly provides written description for pluripotent stem cells derived from non-embryonic sources.

Therefore, coupled with what was well-known in the art at the time of the earliest filing date of the instant application, the specification conveys with reasonable clarity that Applicants were in possession of method for producing embryoid bodies from pluripotent cells...wherein the pluripotent cells are selected from the group consisting of embryonic stem (ES) cells, primordial germ (EG) cells and non-embryonic pluripotent stem cells, within the full scope of the claims. Consequently, Applicants submit that the pending claims fully meet the written description requirements of 35 U.S.C. § 112, first paragraph.

Thus, Applicants respectfully request withdrawal of the rejection of the claims under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement.

III. The Rejection of Claims 1-11, 13, 17, 19-30, 45-52, 54-66, 69 and 70 under 35 U.S.C. § 112, First Paragraph, Enablement, Should be Withdrawn

The Office has rejected claims 1-11, 13, 17, 19-30, 45-52, 54-66, 69 and 70 under 35 U.S.C. 112, first paragraph, as lacking enablement. In particular, the Office alleges that the claims

> ...while being enabling for producing embryoid bodies (EBs) from pluripotent embryonic stem (ES) cells, embryonic germ (EG) cells or *pluripotent non-embryonic stem cells*, does not reasonably provide enablement for a method for producing embryoid bodies (EBs) from multipotent cells, including early primitive ectoderm-like cells, multipotent adult progenitor cells, adult neural stem cells, adult mesenchymal stem cells, ductal stem cells, muscle derived stem cells, hematopoietic stem cells, pancreatic stem cells, follicular stem cells, and any other type of adults stem cells or progenitor cells, and the production of a differentiated cells which is a cardiomyocyte from said EBs.

Office Action at paragraph spanning pages 3-4. (emphasis added). The Office further alleges that from "[a] search of the state of the art of generating EBs, it is apparent that only pluripotent embryonic stem cells or embryonic germ cells can produce EBs under a certain culturing condition" and that there is no evidence of record that demonstrate formation of EBs from various pluripotent adult stem cells in vitro or in vivo. *Id.* at page 5, first paragraph. Applicants respectfully disagree and traverse.

Nonetheless, without acquiescing to the rejection, independent claims 1 and 45 have been amended herein to delete the phrase "pluripotent adult stem cells" and add the phrase "pluripotent non-embryonic stem cells." Therefore, the rejection of the claims under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement has been rendered moot.

With regard to the newly amended claims, Applicants assert that these claims are fully enabled in that they allow one of skill in the art to make and use the invention as claimed without undue experimentation. Furthermore, Applicants note that the Office has conceded on the record that the claims are "enable[ed] for producing embryoid bodies (EBs) from pluripotent embryonic stem (ES) cells, embryonic germ (EG) cells or *pluripotent non-embryonic stem cells.*" Office Action at page 3, paragraph no. 5. To the extent that the newly amended claims may be rejected as lacking enablement, Applicants respectfully disagree and traverse.

The MPEP indicates that "[t]he amount of guidance or direction needed to enable the invention is inversely related to the amount of knowledge in the state of the art as well as the predictability in the art." MPEP §2164.03. "The more that is known in the prior art about the nature of the invention, how to make and use the invention, and the more predictable the art is, the less information needs to be explicitly stated in the specification." *Id.* The MPEP teaches that "predictability or lack thereof" in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. If one skilled in the art can readily anticipate the effect of a change within the subject matter to which the claimed invention pertains, then there is predictability in the art. *Id.*

Claims 1 and 45, as amended herein, are directed to a method for producing embryoid bodies from pluripotent embryonic stem (ES) cells, primordial germ (EG) cells and non-embryonic pluripotent stem cells by obtaining a liquid single cells suspension culture of pluripotent cells, obtaining and suspending the cells in a cell culture container, rocking the container in order to generate cell aggregates, and diluting the suspension and further rocking the container until the cell suspension forms embryoid bodies. Accordingly, the specification, as filed, provides clear and unambiguous evidence of a method for producing embryoid bodies from

pluripotent ES cells, EG cells and non-embryonic pluripotent stem cells and thus renders the claims fully enabled.

The specification provides and the Office concedes that specific guidance on how to use a method for producing embryoid bodies from pluripotent embryonic stem (ES) cells, primordial germ (EG) cells and non-embryonic pluripotent stem cells. In particular, Examples 1 and 2 provide methods for generating embryoid bodies from high and low density cell suspensions and differentiation of cardiac cells. Consequently, the skilled artisan could, without undue experimentation, based on the information provided in Examples 1 and 2, produce embryoid bodies from pluripotent ES cells, EG cells, *and* non-embryonic pluripotent stem cells.

In support of the enablement of the present claims, Applicants respectfully direct the Examiner's attention to the Experimental Report, submitted herewith as Exhibit A, which was generated by Eugen Kolossov, an inventor of the present application. The Experimental Report describes how human induced pluripotent stem (hiPS) cells, which are non-embryonic pluripotent stem cells, were cultured and used to prepare embryoid bodies according to the claimed methods as provided in the Example 2 of the specification.

As indicated in the Experimental Report, after 48 hours, about 5×10^3 EBs per 1×10^6 inoculated iPS cells could be generated. Furthermore, at day 6, EBs generated from human iPS cells could be plated onto fibronectin coated tissue culture plates and further differentiated into cardiomyocytes.

Accordingly, the skilled artisan could, based on the information provided in the specification combined with what was known in the art, produce embryoid bodies from ES cells, EG cells, and non-embryonic pluripotent stem cells using the claimed methods without undue experimentation. Consequently, the claims are fully enabled by the specification as filed and

therefore Applicants respectfully request withdrawal of the rejection of the claims under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement.

III. Conclusion

In view of the foregoing amendments and remarks, Applicant respectfully requests reconsideration and reexamination of this application and the timely allowance of the pending claims. The Examiner is invited to telephone the undersigned if that would be helpful to resolving any issues.

It is believed that no fees are due; however, the commissioner is authorized to charge any fees and credit any overpayments to Deposit Account No. 50-5071. Additionally, please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 50-5071.

Respectfully submitted,

Dated: October 19, 2011

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Experimental Report

Here we demonstrate the suitability of the method described in the international application WO 2005/005621 to produce "Embryoid bodies" (EBs) from *human pluripotent stem cells; i.e.* human induced pluripotent stem cells (iPS cells), and to differentiate these cells within the EBs to cardiomyocytes. As acknowledged in the literature human iPS cells exhibit the same properties as naturally occurring human pluripotent stem cells and may therefore be used as an adequate substitute in the generation of "Embryoid bodies".

Human iPS (hiPS) cells generated from fibroblasts [1-3] were cultured as described elsewhere [1; 4] (Fig. 1). hiPS cells were trypsinized to obtain a single cell suspension, and differentiation was started (= d0) using the single cell suspension culture with a concentration of 1-5x10^5 iPS cells per ml in a total volume of 5 ml (preferentially 2x10^5 hiPS cells/ml, 1x10^6 hiPS cells per 5 ml) in an appropriate container, e.g. a 6 cm Petri dish or one well of a standard 6-well plate. The preparation of EBs essentially followed protocol 2 as described in WO 2005/005621 at page 10, line 32 to page 11, line 8 and illustrated in Example 2. Thus, the cell suspension in the container (either a Petri dish or a 6-well plate) was incubated on a rocking table at about 50 rpm for 48 hours. Within this time, about 5x10^3 EBs per 1x10^6 inoculated iPS cells could be generated (Fig. 2, A and B).

After 48h (d2), resulting EBs were diluted to a density of 100-200 EBs/ml, and were further cultured in suspension, e.g. in 10 cm Petri dishes (bacterial grade plastic) at a density of 1x10^3 EBs in 10ml)..

At day 6, EBs generated from human iPS cells using the method described above could be cultured further either in suspension as described above (100-200 EBs/ml either in a 10 cm Petri dish or a Spinner flask, see Fig. 3 A), or plated at onto fibronectin coated tissue culture plates (e.g. 40 EBs per 10cm Petri dish) for further differentiation. In both cases, first cardiomyocytes appeared around d21. These cardiomyocytes can be either identified by their spontanous beating activity, or by the expression of a fluorescence reporter gene (e.g. YFP) under control of a cardiac-specific promotor, e.g. the cardiac alpha myosin heavy chain promotor (Fig. 3, B - D).

The above-described method have been used also for differentiating neuronal cells from human iPS cells. To this end, EBs generated by the method described above were plated on fibronectin-coated tissue culture plates at day 6. At day 8, medium was changed to a neuronal differentiation medium. Around d25 first neuronal cells appear in the cultures, which were identified by their morphology as well as by immunostaining for neuron-specific markers, e.g. beta-Tubulin and alpha-Hydroxylase (Fig. 4, A - D).

Cologne, 21 June 2011 — Dr. Eugen Kolossov.

Place, Date

Literature

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(4) Sugii S, Kida Y, Berggren WT, Evans RM. Feeder-dependent and feeder-independent iPS cell derivation from human and mouse adipose stem cells. Nat Protoc. 2011 Mar;6(3):346-58. Epub 2011 Feb 24.